Second Preliminary Amendment USAN 10/712,900 Docket No. 200144.405D1

## **Amendments to the Specification:**

Please replace the paragraph beginning at page 7, line 15, as follows:

Since the finding of differential expression of LPAAT-B mRNA in certain tumor versus normal tissues is based on Northern analysis of a single specimen from a given tissue, more studies will be needed to determine whether the relative elevation of LPAAT-β expression in selected tumor tissues can be applied and extended to similar tissues derived from a larger number of donors. Leung et al., DNA Cell Biol. 17: 377 (1998). Accordingly, in situ hybridization was used to compare LPAAT-\beta mRNA levels in breast, ovary, and prostate tumor samples obtained from multiple independent donors (LifeSpan Biosciences, Seattle, WA). Specifically, the coding region of human LPAAT-β was amplified by PCR from the plasmid LPAAT-β with primers 5'-GCATGAATTC AAAGGCCTAC GTCGACATGG pCE9. AGCTGTGGCC GTG-3' (SEO ID NO:1) and 5'-GTCGACTCTA GACTACTGGG CCGGCTGCAC-3' (SEQ ID NO:2). The resultant 870 bp PCR product was then cut with EcoR I and XbaI for insertion in between the EcoR I and XbaI sites of the in vitro transcription vector pDP18-T7/T3 (Ambion, Austin, TX) to generate the plasmid pDP lptB. Serial tissue sections from paraffin archival samples were hybridized with digoxigenin labeled riboprobes transcribed from either a T3 (sense) or T7 (antisense) transcription initiation site present in the plasmid pDP lptB linearized with either EcoR I (antisense) or Xba I (sense). The tissue sections from paraffin blocks were digested with proteinase K (20 µg/ml) for 4 minutes, then hybridized with the antisense probe (1 µg/ml) at 60° C for 22 hours and subsequently washed with 2xSSC and 0.1xSSC at 50° C. The hybridization signals were detected with NBT/BCIP substrates using three cycles of an alkaline phosphatase TSA amplification system (NEN Life Sciences, Boston, MA). The specimens were then counterstained with methyl green. The signal was developed within 30 minutes at room temperature. The slides were then imaged using a digital camera mounted onto a microscope.

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Please replace the paragraph beginning at page 29, line 22, as follows:

For the construction of Baculovirus expression vectors, the full-length human LPAAT-B cDNA was amplified by PCR from the DNA template pCE9.LPAAT-β (West et al., DNA Cell 16:691-701 (1997)) using the primers 5'- TGATATCCGA AGAAGATCTT ATGGAGCTGT GGCCGTGTC-3' (olpb1F; SEQ\_ID\_NO:3) and 5'-CAGGCTCTAG ACTACTGGGC CGGCTGCAC-3' (olpb1R; SEQ ID NO:4). The ~870 bp fragment generated was reamplified by PCR using the primers 5' CCTACGTCG ACATGGAACA AAAATTGATA TCCGAAGAAG ATC-3' (olpb2F; SEQ ID NO:5) and 5'-CAGGCTCTAG ACTACTGGGC CGGCTGCAC-3' (olpb1R; SEQ ID NO:6). The ~890 bp fragment generated was then cleaved with Sal I and Xba I for insertion into pFastBac™ HTc vector (Life Technologies, Gaithersberg, MD) between the Sal I and Xba I sites for the generation of the plasmid pFB.LPAAT-β. This plasmid was then transformed into E. coli DH10Bac<sup>TM</sup> (Life Technologies, Gaithersberg, MD) for the generation of recombinant Bacmid DNA for transfection into HighFive (Invitrogen, San Diego, CA) or SF9 insect cells for the production of recombinant Baculovirus stocks using the protocol described in the Bac-to-Bac® Baculovirus Expression System (Life Technologies, Gaithersberg, MD), a eukaryotic expression system for generating recombinant baculovirus through site- specific transposition in E. coli. Viral stocks harvested from the transfected cells can then be used to infect fresh insect cells for the subsequent expression of LPAAT-\( \beta \) fusion protein with a poly-histidine tag and a myc-epitope near its N-terminus. The membrane fraction from these Sf9 cells would be the source of LPAAT enzyme.

Please insert the enclosed paper entitled "Sequence Listing" immediately after the section of the specification entitled "Abstract of the Disclosure" on page 49.

## **Amendments to the Drawings:**

The drawings have been corrected as requested in the Formalities Letter dated March 14, 2004.

Attachment: 21 Replacement Sheets - Figs. 1-9